RATE STUDIES OF TRILAURIN HYDROLYSIS BY PANCREATIC LIPASE

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INTRODUCTION

Milk is usually purchased because of its flavor. The absence of normal flavors or the presence of undesirable ones therefore could result in economic losses to the dairy industry. Rancidity is one objectionable milk flavor which, if it could be controlled, would result in considerable savings for the industry.

It has been reported that certain short chain fatty acids caused rancid flavors. These acids, apparently resulted from the action of an enzyme (lipase) on the triglycerides of milk fat.

The structure and properties of lipase or its substrate, milk fat, are not completely known. Neither is the mechanism of their reaction with each other completely understood. Lipase is usually present in all raw milk in sufficient quantities to cause rancidity, but all milk does not become rancid at the same rate. This difference in reaction rates may be due to variations in such reaction conditions as: enzyme concentration, substrate concentration, time, temperature or pH.

Although the individual influence of these factors on lipase hydrolysis of triglycerides has been studied, their relative importance is not known. The possibility that interactions between these factors might affect the

reaction rate also has not been explored.

Certain treatments which the milk may undergo have been reported to markedly change the rate of rancid flavor development. Some of these treatments (temperature changes and agitation, for example) are quite likely to have changed the physical state of the milk fat. Thus, the rate of rancid flavor development could be governed in part by changes in the physical nature of milk fat which make the substrate more or less available to the enzyme.

A study of the physical properties of milk fat triglycerides and the interactions involved between temperature, enzyme concentration and substrate concentration as they affect this hydrolysis could be a step in the direction of controlling rancid flavors.

The objectives of this research were: (a) To determine what influence changes in the physical state of milk fat might have on the rate at which this substrate was attacked by lipase; (b) To evaluate any interactions which occurred between enzyme concentration, substrate concentration and temperature as they influenced lipase hydrolysis of milk fat; (c) To determine the relative importance of each variable as they influenced this reaction.

Since milk is a complex substance, the reaction was first studied in a simplified model system involving pancreatic lipase and a simple triglyceride (trilaurin). The study of this model system was the subject for this thesis.

REVIEW OF LITERATURE

The literature concerning rancid flavors and their causes is voluminous and several good reviews of the subject are available (4, 11, 24, 28). The review in this thesis was not intended to cover the entire subject of rancidity, but to deal only with those factors which may govern the rate at which rancid flavors develop.

Esterases are enzymes which catalyze the hydrolysis of esters. Those that preferentially hydrolyze the esters known as triglycerides are called lipases. Esterases and lipases are widely distributed in nature, being found in microorganisms, the seeds of higher plants and in many animal tissues -- particularly in the liver and pancreas (14).

Tarassuk and Frankel (27) have reported that cows milk contained at least two different lipase systems. One, "naturally active lipase," was thought to be irreversibly adsorbed on the fat-globule when freshly drawn milk was cooled. The other was the "lipase of normal milk," which remained in the plasma and was associated with the caseinate fraction. Nilsson and Willart (23) reported that most milk contained enough lipase to cause it to go rancid, but no fatty acids were formed until the substrate had been made available to the enzyme.

According to Nachlas and Seligman (22), the ideal substrate for pancreatic lipases were the glycerides of long chain fatty acids, and for esterases the simple esters of short chain carboxylic acids were the ideal substrates. Both groups of enzymes split esters of intermediate sized chains. These authors found that the pancreas (which they believed to contain both esterases and lipases) of six animal species, hydrolyzed three substrates prepared from β-naphthol and C_2, C_{12} and C_{16} - C_{18} fatty acids.

Milk lipases (10, 15) and pancreatic lipases (3, 4, 19, 20) were thought by various investigators to preferentially attack the primary, or alpha, linkages of triglycerides. Mattson and Beck (20) further found that the hydrolysis of triglycerides by pancreatic lipase was a series of directed stepwise reactions proceeding from triglycerides through 1, 2 diglycerides to 2 monoglycerides. They found that the reaction followed the same route, whether the glycerides contained palmitic, stearic or oleic acids. Borgstrom (4) reported that the hydrolysis of glycerides by rat pancreatic lipase proceeded via the 1, 2 diglyceride to both 1 and 2 monoglycerides, but the 2 form predominated. Balls and Matlack (3) stated that "it is safe to assume that the splitting of a secondary ester linkage takes place at an almost negligible velocity in comparison with the splitting of a primary ester linkage." Harwalkar and Calbert (11) also found a slight degree of selectivity for the short-chain fatty acids upon extensive

hydrolysis of milk fat by milk lipase.

Activation treatments such as temperature fluctuations, agitation and homogenization were thought by some workers (6, 12) to alter the substrate thereby inducing lipolysis (hydrolysis of an ester by lipase). Others (28) have thought that activation treatments produced a change in the lipase system.

The rate at which milk fat was hydrolyzed was influenced by: the "activation" procedure, the enzyme concentrations, the substrate concentration, the fat-plasma ratio, the physico-chemical properties of the fat and the conditions of the fat interface (5, 12, 17, 18, 22, 26). Krukovsky and Sharp (17) found that the lower the temperature required to crystallize a fat fraction, the greater the increase in acidity, when this fraction was used as a substrate for milk lipase. These results indicated that the rate of lipolysis was dependent upon the melting point of the fat or upon the amount of crystalline fat at any given temperature. Similarly, Entressangles et al. (7) reported that tristearin was hydrolyzed slowly and believed this was because tristearin was solid at the experimental temperatures used.

Tarassuk and Frankel (26) stated that lipolysis was a surface reaction and was activated by the formation of foam. They theorized that foam provided optimum conditions for the reaction by (a) increasing the air-liquid interface area, (b) concentrating the enzyme at this interface, (c) activating the substrate by surface denaturation of the fat globule, and (d) causing intimate contact of the enzyme and activated substrate.

Krukovsky and Sharp (18) found that the amount of lipolysis, induced by shaking, had little or no relation to a cow's breed, milk production, or to the season of the year. The extent of the reaction was attributed rather to an alteration in the surface character of the fat globules which created a condition more favorable for lipolysis.

The rate of lipolysis also has been found to be affected by pH and the presence of calcium ions. Desnulle, Naudet and Rouyier (5) found that triglycerides and diglycerides were rapidly hydrolyzed, at a pH of 8.0 in the presence of one Ca^{++} ion per liberated fatty acid chain. Mattson and Beck (19) stated that Ca^{++} ions prevented the synthesizing of new triglycerides from liberated fatty acid chains. Schwartz, Gould and Harper (24) found the optimum pH for milk lipase was 8.5 and Borgstrom (4) reported an optimum pH of 8.0 for pancreatic lipase.

Nachlas and Seligman (22) reported that Willstatter and Menmen found the addition of 2×10^{-2}M sodium taurocholate (a bile salt) accelerated the hydrolysis of methyl butyrate and triacetin by pancreatic lipase. Mattson and Beck (19) found that the addition of low concentrations of bile salts slightly activated the hydrolysis of triglycerides, containing palmitic, stearic or oleic acid. Higher concentrations of these salts inhibited the hydrolysis.

Lipase has been inactivated by various treatments including heat, acid, alkali, and light (8, 9, 13).

According to Fodor (8), the inactivation of lipase by heat and alkali had a different influence on mono and diglycerides than it had on triglycerides. The hydrolysis of mono and diglycerides was almost complete in the presence of heat, but triglyceride hydrolysis was much slower and less complete at the same temperature. Alkali caused a decreased amount of mono and diglyceride hydrolysis, but it had little effect on the hydrolysis of triglycerides.

Frankel and Tarassuk (9) concluded that the heat inactivation of milk lipase followed a first-order reaction, and that only the enzyme activity was changed in relation to time. These workers also found that the acid and alkali inactivation of milk lipase was not changed by the presence or absence of milk fat globules. They also reported that "the photo-inactivation of milk lipase appeared to be an autocatalytic reaction."

A considerable portion of the loss in lipase activity of some milk aged at low temperatures has been traced to oxidation of the enzyme, and specifically to oxidation of the sulfhydryl group (9).

Herrington and Krukovsky (13) found that lipase activity was retarded by rapid cooling of milk. They cooled the milk from 38°C. to 3.5°C. in less than 30 seconds. However, Johnson and Von Gunten (16) found that milk which was cooled immediately (to 32°F in about ten minutes) developed a greater rancid flavor than the same milk left at atmospheric temperature until milking was completed and

then cooled by the same method. According to Jenness and Patton (14) the lipase activity of fresh milk decreased rapidly on holding to the extent that as much as half of the activity was lost in 3 hours at 37°C. or in 48 hours at 0 to 5°C.

Jenness and Patton (14) found that milk lipase activity was decreased as a result of heat treatments and that exposure to 50°C. for 15 minutes reduced the lipase activity by as much as one half. They found also that milk lipase was readily inactivated by exposure to sunlight.

In summary, it has been shown that lipases preferentially attack the primary linkages of triglycerides and that these lipases preferentially attack short chain triglycerides. There has been general agreement that heat, acid, alkali, light or a combination of these factors will inactivate lipases. Lipolysis can be activated by temperature fluctuations, agitation or homogenization. Disagreement exists about how activation treatments change the reaction. Some workers have thought that the lipase system was affected, while others reported that the treatments change the substrate.

EXPERIMENTAL METHODS

In this work, a simple triglyceride, trilaurin[1], was subjected to the action of pancreatic lipase[2] while varying the enzyme concentration, the substrate concentration, and the reaction temperature. A series of 24 trials were conducted using a 6 x 2 x 2 factorial design. Six temperatures (30-55°C at 5°C intervals), two levels of substrate (4.65×10^{-3}M and 9.3×10^{-3}M) and two levels of enzyme concentration (0.4% and 0.8%) were used in this experiment. Eight duplicate trials were selected at random. The sequence in which the 32 trials were conducted was assigned at random (25).

The reaction mixtures were as follows:

3.0 ml (45%) $CaCl_2$
37.5 ml (0.1M) "Tham" (Tris-hydroxy-methyl-amino-methane)
(4.65×10^{-3}M or 9.3×10^{-3}M) Trilaurin
0.075 or 0.15 g Sodium choleate (bile salts), corresponding to the low or high substrate concentrations.
(0.4% or 0.8%) Enzyme concentration.
Plus, sufficient water to bring the total volume to 150 ml.

The Trilaurin, sodium choleate, $CaCl_2$, "tham" and 50 ml of water were combined in a hand operated laboratory homogenizer. After homogenizing the mixture, sufficient

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[1]Eastman Kodak Co., Dallas 35, Texas.

[2]Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

distilled water was used to rinse the apparatus and bring the total volume to 135 ml. The beaker containing the mixture was then placed in a water bath, at the reaction temperature, and the mixture was allowed to come to equilibrium with the bath temperature. During this time the pH of the mixture was adjusted to 8.0 by the addition of HCl.

Fifteen ml of the enzyme suspension were then placed in a graduated cylinder which also was adjusted to the water bath temperature. On reaching this temperature, the enzyme suspension was added to the digestion beaker. The pH was readjusted to 8.0, if necessary, by adding 0.1 N NaOH and the reaction began.

The pH of the mixture was maintained at 7.8-8.0 during the remainder of the digestion period (60 minutes) by adding NaOH as necessary.

The lauric acid released during the reaction was determined by measuring the added NaOH. In addition, two -25 ml aliquots of the reaction mixture were removed at 10, 20 and 60 minutes and the reaction was stopped by reducing the pH to less than 2.0 with H_2SO_4.

The fatty acids in the samples were extracted with the following procedure:

(a) Twenty-five ml of ethanol were added and the mixture was shaken 10 times in a separatory funnel.

(b) Seventy-five ml of ethyl ether and 25 ml of petroleum ether were then added. This mixture was shaken

for 45 seconds before the ether layer was decanted.

(c) The extraction (part b) was repeated twice.

The three ether extracts were combined and titrated with 0.2N alcoholic potassium hydroxide[3] using alcoholic phenolphathein as an indicator.

Controls (reagent blanks) were prepared by boiling the lipase solution to inactivate it before it was added to the substrate. The incubation period for these controls was omitted.

The objective in obtaining two sets of data was to use one as a check on the other. It was found, however, that the two methods were only comparable during the first 30 minutes of the reaction period. During the last 30 minutes the titration values were considerably higher than those obtained by extraction. To check the extraction procedure, lauric acid was added to a digestion mixture. More than 95% of this added acid was recovered by extraction, and on this basis the extraction procedure was considered to be accurate.

Since titration data were available during the first 10 minutes of the reaction, reaction rates were obtained from these data. The rates were slopes from graphs of the log reaction time (0 - 10 minutes) vs. log lauric acid hydrolysis. The data obtained by extraction were used to evaluate the remainder of the reaction. Values for both

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[3]The alcohol was distilled before use.

methods were expressed as $\frac{a}{a-x}$, where "a" = 100% hydrolysis and "x" = the % hydrolysis at any given time.

Both sets of data (reaction rates and hydrolysis at 10, 20, and 60 minutes) were analyzed by an analysis of variance technique. The mean squares were used to determine the relative importance of each variable and the significance of any interaction between variables.

To determine the solidification point of trilaurin, suspensions of this triglyceride in water were heated until the trilaurin became liquid. These suspensions were then placed in a double walled, silvered vacuum tube which was equipped with a thermistor (sealed in glass) and a vertical stirrer. The apparatus was similar to that described by Meir, Glasgow and Rossini (21). The trilaurin suspensions were stirred at a rate of 120 strokes per minute, while being cooled at selected rates. The electrical resistance of the thermistor was measured with a wheatstone bridge and a galvanometer. These resistance values were then converted to temperatures by comparing them to curves made from data relating the resistance of water to its temperature. The solidification point of trilaurin was determined by finding the point in the temperature vs time curve where the cooling rate was slowed by the heat of crystallization.

RESULTS AND DISCUSSION

The data (Tables I and II) are more easily interpreted when presented as drawings such as those graphed in Figures 1-6. The greatest reaction rate occurred at 40°C. (Table II), and the optimum temperature for this reaction apparently was between 40 and 45°C. (Figures 1, 2, 3 and 6). This agreed with the 40°C. temperature used by Mattson and Beck (20).

The increased reaction rate between 30°and 40° ($30^{\circ} < 35^{\circ} < 40^{\circ}$) was thought to be due to increased enzyme activity as a result of the increased temperature. Such results were expected and were in agreement with those of Jenness and Patton (14). The decreased reaction rates above 45°C. ($45^{\circ} > 50^{\circ} > 55^{\circ}$) also were thought to be caused by temperature. In this case the heat appeared to partially inactivate the lipase.

Figures 2 and 3 indicated that the amount of hydrolysis generally increased with increased enzyme and substrate concentration. In Figure 3, the lower level of substrate appeared to give the greatest response; however, these data were based on the percent hydrolysis and not on the volume of substrate hydrolyzed. In absolute values there was more hydrolysis at the higher substrate level than at the lower level.

The reaction increased with time and appeared to follow an exponential curve (Figure 4). When graphed as the log of trilaurin hydrolysis vs time the reaction appeared to proceed at three separate rates (Figure 5). The fastest of these three rates may have represented the hydrolysis of only the alpha, or primary linkages. This rate occurred during the first 10 minutes of the reaction. In the next time period the reaction rate was somewhat slower. This may have represented hydrolysis of both alpha and beta, or secondary linkages. The slowest hydrolysis rate could have represented only hydrolysis of the beta linkages. This change in rates during the reaction was another reason the slope data were calculated during the first 10 minutes when the reaction was assumed to be of the first order.

The statistical analyses of the data evaluated all three measured variables as they influenced the trilaurin hydrolysis. The calculation, an analysis of variance technique, evaluated a four dimensional surface which represented temperature, substrate concentration and enzyme concentration, as they influenced lauric acid formation (the reaction response). The analyses also evaluated the relative importance of each variable and determined whether there were any interactions between them. To the authors knowledge, information of this nature has not previously been available for reactions of this type.

All four dimensions of the actual surface could not be pictured, but examples of the data have been graphed as

three dimensional surfaces in Figure 1, 2 and 3.

When the rates for the first ten minutes of the reaction were analyzed (Table III), the substrate level was of greatest importance as shown by the relative size of the mean square values. This would seem to indicate that at the low substrate concentration the rate was slower due to a lack of substrate but the rate remained constant when the higher substrate concentrations were used. Both enzyme concentration and temperature were statistically significant.

There was a significant temperature x enzyme interaction which indicated that the combination of the two variables had an effect in addition to that accounted for by each variable acting separately. All or part of this effect could have been due to increased enzyme activity because of the increased temperature.

There also was a significant temperature x substrate interaction which could have been due to temperature changes causing a change in the physical state of the substrate. Some workers have indicated that there may exist a difference in the rate at which solid and liquid triglycerides are attacked (7). However, this could not be visibly demonstrated. To do so, one would have to have produced a change in the physical state of the substrate without changing the temperature, pressure or any other variable which influenced the reaction. This of course would not be physically possible.

The solidification temperature of the trilaurin was near the temperature at which inactivation of the lipase began. Therefore, it was possible that any increased activity due to a change in the physical state of the trilaurin would have been counterbalanced by a decreased enzyme activity. Since these two factors could not be physically separated, the importance of each factor could not be demonstrated except by statistical analysis.

The highly significant enzyme x substrate interaction could also result partly from a change in the physical state of the substrate. The increased or decreased enzyme activity which occurred with temperature change may also have been responsible, in part, for this interaction. It is interesting to note that this interaction was not significant during the later stages of the reaction (Table IV).

The non-significant temperature x enzyme x substrate interaction indicated that these three variables together contributed nothing to the reaction in addition to what had already been measured.

The relative size of the mean square values for the extraction data (Table IV) showed that temperature was the most important single factor influencing this reaction during its later stages. These data more nearly fitted a quadratic or exponential equation than a linear one.

The enzyme concentration was next in importance and the substrate concentration was least important of these three.

There was a highly significant temperature x enzyme interaction. With one exception, the temperature x substrate interaction was not significant. Thus, in this case there was almost no additional effect when the two variables acted together, as compared to the effect when each acted separately.

The "lack of fit" term in Table IV included the temperature x enzyme x substrate interaction, plus other variables which had to be deleted because no program was available for the IBM 650, which would handle a matrix of the size involved (43 x 43).

To determine what influence the rate of cooling had on the temperature at which trilaurin solidified, the triglyceride was cooled at three different rates using two concentrations of trilaurin in water (Table V).

When cooled in a concentrated state (39%) and at a slow rate (0.17°C per minute) trilaurin had an average solidification temperature of 43.9°C, which was near the 46.4°C melting point for the beta crystal form of this triglyceride. When cooled in a dilute solution (4.0%) and at a fast rate (2.7°C per minute), the triglyceride solidified at 35.5°C. This could have been the beta prime crystal form, which melts at 35.0°C (2). The data indicated that the solidification temperature of trilaurin decreased as the rate of cooling increased and as the concentration of the triglyceride in water decreased. The data further indicated that the solidification temperature of trilaurin

in the digestion mixtures used in this work was probably between 35° and 40°C, since dilute mixtures of the substrate (0.3 and 0.6%) were used.

If lipase acted faster on a liquid substrate than a solid one (which was shown to be a possibility) this change in solidification temperature with variations in cooling rates could partially explain why rapidly cooled milk sometimes has become rancid faster than milk which was cooled slower (16). The fat in rapidly cooled milk may have remained liquid longer than similar fat in slowly cooled milk.

SUMMARY

The rate at which trilaurin was hydrolyzed by pancreatic lipase was studied in a simplified model system. The relative importance of reaction temperature, enzyme concentration and substrate concentration as they influenced the reaction were evaluated. In addition, the significance of interactions between these variables was determined.

The experiment was conducted in a series of 32 trials which included eight duplicates. Temperatures were varied between 30 and 55°C (in 5.0°C intervals). Enzyme concentrations were 0.4 and 0.8% and substrate concentrations were 4.65×10^{-3}M and 9.3×10^{-3}M.

Statistical analysis showed each factor was significant when evaluated independently. In addition, the temperature x enzyme, temperature x substrate and enzyme x substrate interactions were statistically significant during the first ten minutes of the reaction. In the later stages of the reaction only the individual factors and the temperature x enzyme interaction were statistically significant.

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A P P E N D I X

TABLE I

TRILAURIN HYDROLYSIS AT 10, 20 AND 60 MINUTES OBTAINED BY THE EXTRACTION METHOD AND EXPRESSED AS $\frac{a}{a-x}$ [a]

		465 x 10^{-3}M			9.3 x 10^{-3}M[b]			
		0.4		0.8	0.4		0.8[c]	
Temp.°C.	Minutes							
30°	10	1.23		1.24	1.13		1.27	
	20	1.43		1.51	1.24		1.43	
	60	2.11		2.50	1.44		1.95	
35°	10	1.48	1.53	1.87	1.56		1.45	
	20	1.69	1.89	2.50	2.11		1.92	
	60	2.85	3.41	4.86	3.43		3.25	
40°	10	2.24		2.21	1.94	2.00	2.43	
	20	3.24		3.29	2.75	2.44	3.71	
	60	5.98		6.51	4.62	3.95	5.71	
45°	10	1.83	1.61	2.30	1.52	1.23	1.83	2.02
	20	2.47	1.89	3.75	1.74	1.54	2.43	2.64
	60	3.14	2.91	5.58	2.12	2.13	3.71	5.41
50°	10	1.05		1.37	1.13	1.20	1.16	
	20	1.18		1.64	1.23	1.29	1.28	
	60	1.29		1.98	1.37	1.40	1.42	
55°	10	1.02		1.21	1.08	1.06	1.05	1.08
	20	1.03		1.33	1.09	1.07	1.07	1.10
	60	1.07		1.41	1.19	1.15	1.16	1.23

[a] a = 100% and x = the % hydrolysis at any given time.

[b] Substrate concentration expressed as molarity.

[c] Enzyme concentration expressed as % by weight.

TABLE II

REACTION RATES AS SLOPES CALCULATED FROM A LINE DRAWN THROUGH THE 0-10 MINUTE DATA FROM THE TITRATION METHOD[a]

	4.65×10^{-3}M		9.3×10^{-3}M[b]	
Temp. °C.	0.4	0.8	0.4	0.8[c]
30	1.50	2.13	1.10	1.25
35	2.10	6.00	1.80	2.00
	2.40			
40	4.20	8.33	3.19	3.50
			2.80	
45	2.60	7.00	2.00	3.60
	4.00		1.20	1.97
50	1.19	1.50	1.08	1.18
			1.36	
55	1.06	1.20	1.05	1.10
			1.10	1.00

[a]Calculated from log time vs. log $\frac{a}{a-x}$, where a = 100% and x = the % hydrolysis at any given time.

[b]Substrate concentration expressed as molarity.

[c]Enzyme concentration expressed as % by weight.

TABLE III

THE SOURCE, DEGREES OF FREEDOM AND MEAN SQUARES FROM AN ANALYSIS OF VARIANCE OF SLOPE VALUES CALCULATED FROM 0-10 MINUTE DATA OBTAINED BY THE TITRATION METHOD

Source	df	Mean Squares
Total	32	
Mean	1	
Temperature	5	9.136[a]
Enzyme	1	10.082[a]
Substrate	1	14.828[a]
Temperature x Enzyme	5	1.647[b]
Temperature x Substrate	5	2.043[b]
Enzyme x Substrate	1	6.029[a]
Temperature x Enzyme x Substrate	5	0.792
Duplicates	8	0.349

[a]Significant at the 1% probability level.

[b]Significant at the 5% probability level.

TABLE IV

THE SOURCE, DEGREES OF FREEDOM AND MEAN SQUARES FROM AN ANALYSIS OF VARIANCE OF THE EXTRACTION DATA FOR 10, 20 AND 60 MINUTES[a]

Source	df	Min. 10	Min. 20	Min. 60
Total	32			
Mean	1			
Temperature (linear)	1	0.596[b]	2.080[b]	14.642[b]
Temperature (quadratic)	1	2.860[b]	9.244[b]	34.613[b]
Enzyme	1	0.382[b]	1.693[b]	7.588[b]
Substrate	1	0.068[c]	0.329[c]	1.775[c]
Temp. linear x Enzyme	1	0.002	0.000	0.029
Temp. quadratic x Enzyme	1	0.116[c]	0.621[b]	2.730[b]
Temp. linear x Substrate	1	0.000	0.005	0.402
Temp. quadratic x Substrate	1	0.042	0.205[c]	0.653
Enzyme x Substrate	1	0.013	0.113	0.267
Temp. x Enzyme x Substrate plus Lack of Fit	14	0.107[c]	0.372[b]	1.516[c]
Duplicates	8	0.011	0.035	0.232

[a]Trilaurin hydrolysis expressed as $\frac{a}{a-x}$, where a = 100% and x = the % hydrolysis at any given time.

[b]Significant at the 1% probability level.

[c]Significant at the 5% probability level.

TABLE V

SOLIDIFICATION TEMPERATURES OF TRILAURIN[a] IN WATER COOLED AT SELECTED RATES

% Trilaurin in H_2O	Cooling Rates (°C./min.)		
	0.17[b]	1.2[b]	2.7[b]
4	40.7	38.0	33.7
4	40.6	38.4	37.2
39	44.0	43.0	40.0
39	43.8	43.2	42.4

[a]Cooled in a -78°C. Dry ice and Ethanol bath.

[b]These rates were obtained between 40 and 47°C from curves of temperature vs time.

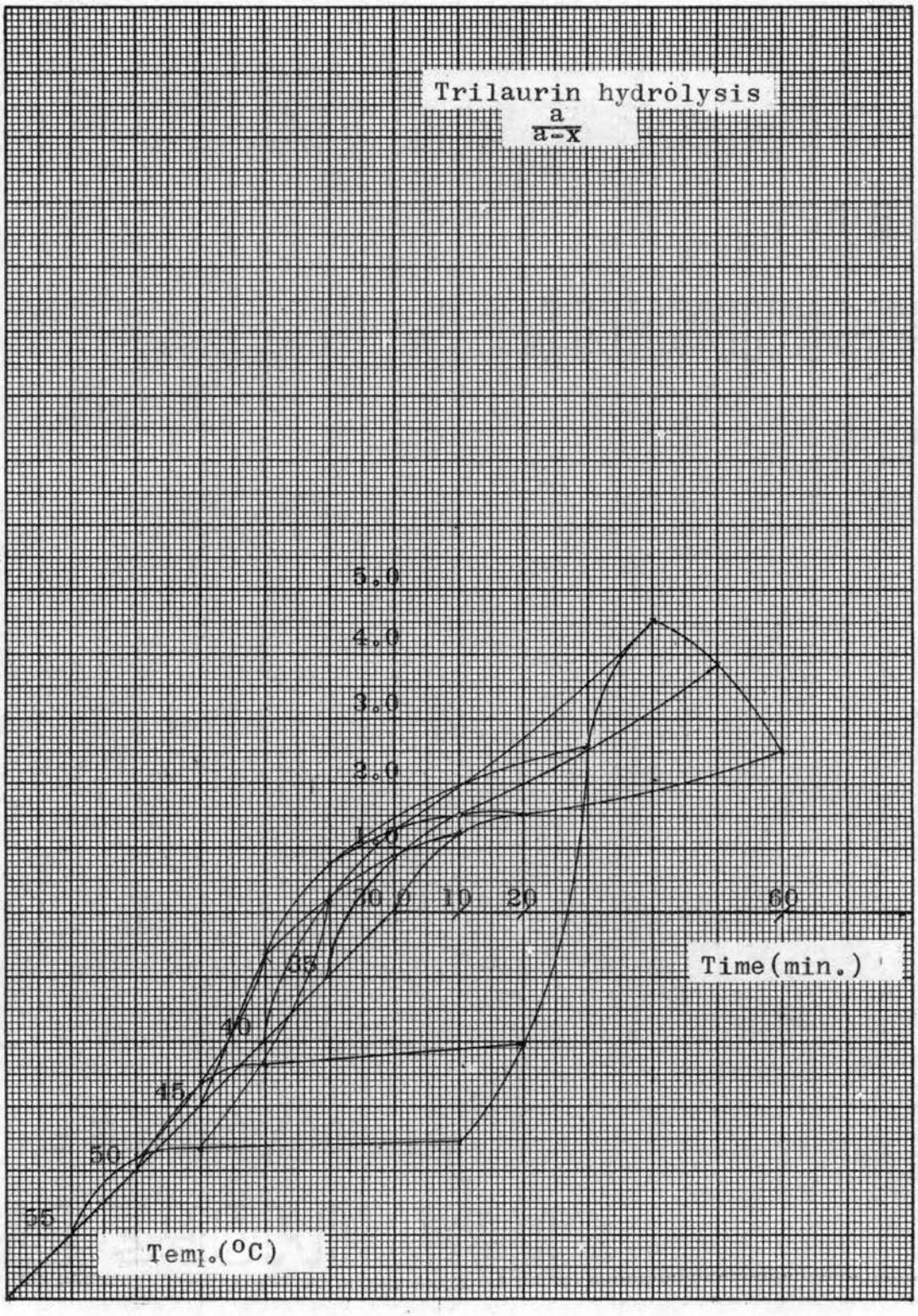

Figure 1. A Surface Representing Trilaurin Hydrolysis as Influenced by the Reaction Time and Temperature When Enzyme and Substrate Concentrations were 0.8% and 4.65 x 10^{-3} Molar, Respectively.

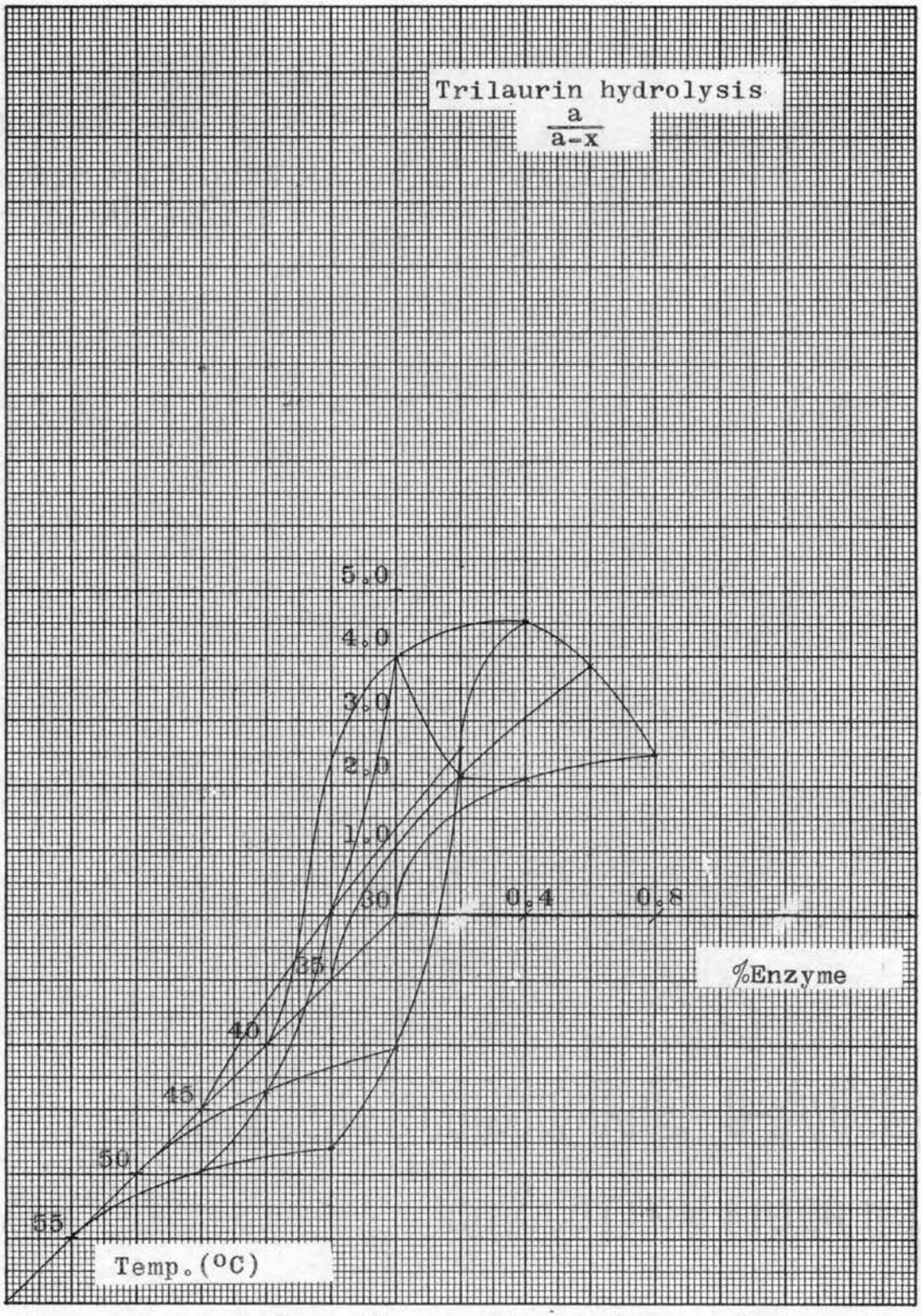

Figure 2. A Surface Representing Trilaurin Hydrolysis as Influenced by the Reaction Temperature and Enzyme Concentration, after 60 Minutes Digestion with a 4.65×10^{-3} Molar Substrate Concentration.

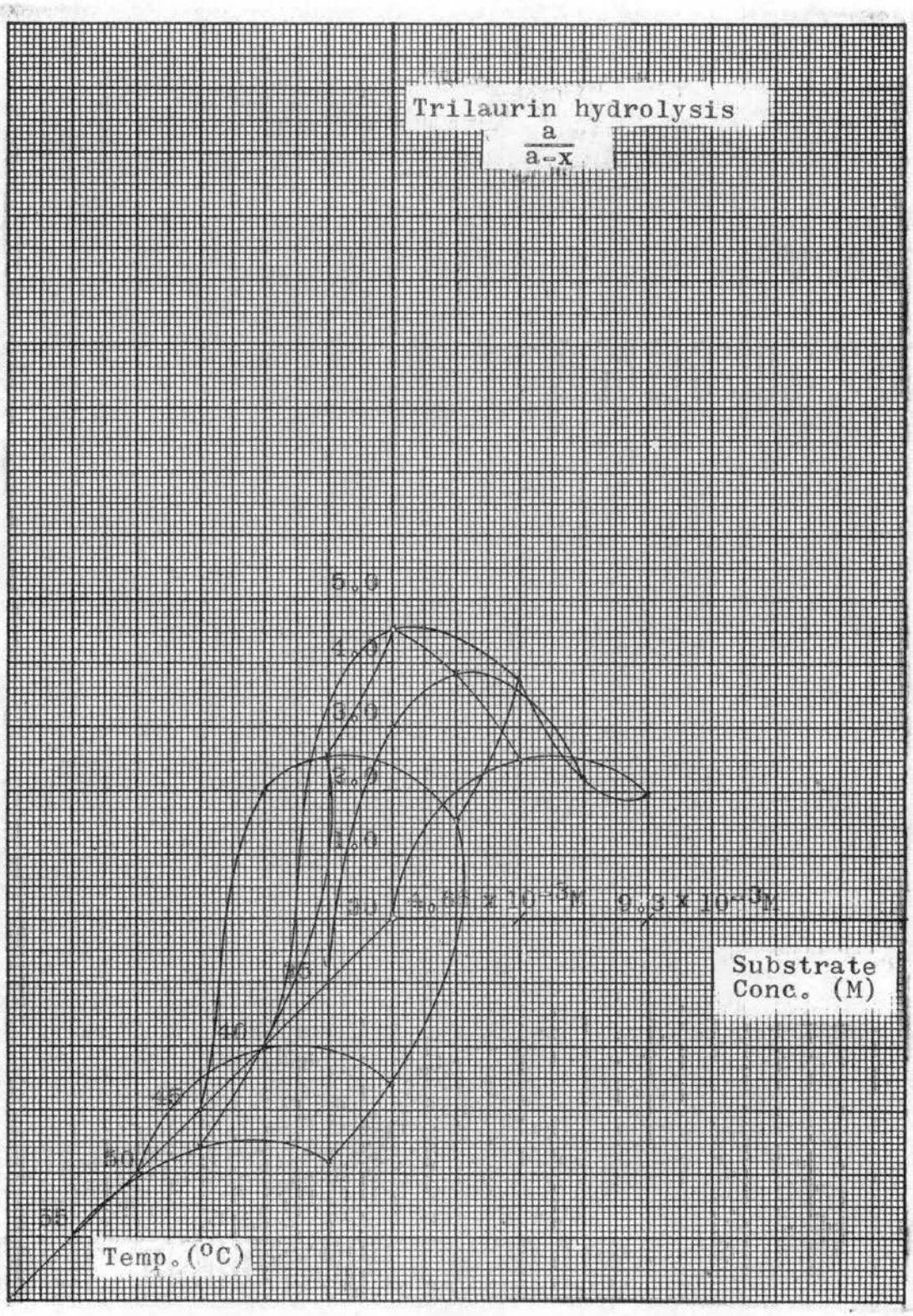

Figure 3. A Surface Representing Trilaurin Hydrolysis as Influenced by the Reaction Temperature and Substrate Concentration, after 60 Minutes Digestion with a 0.8% Enzyme Concentration.

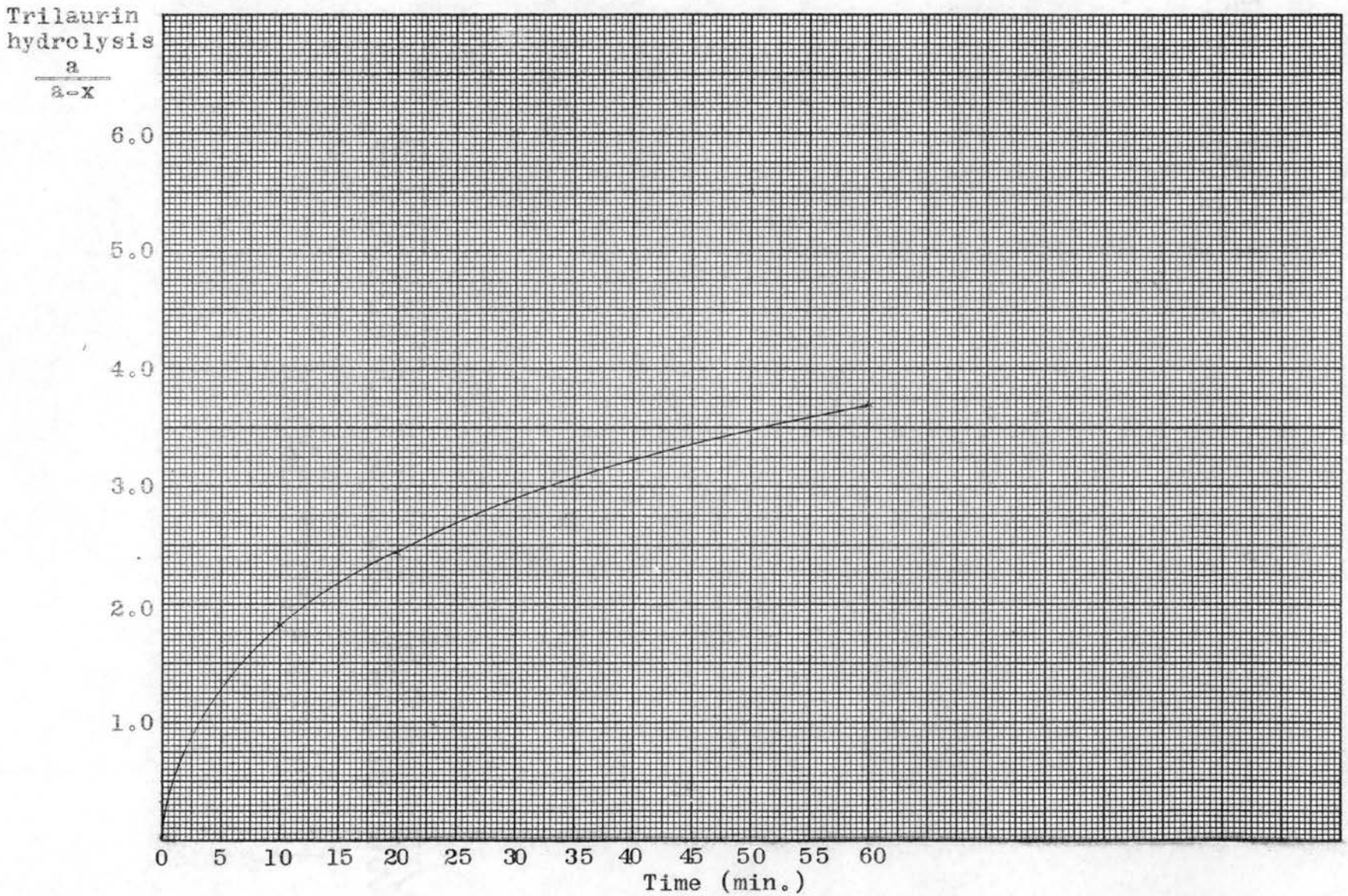

Figure 4. A Typical Reaction Curve of Trilaurin Hydrolysis ($\frac{a}{a-x}$) vs Time.

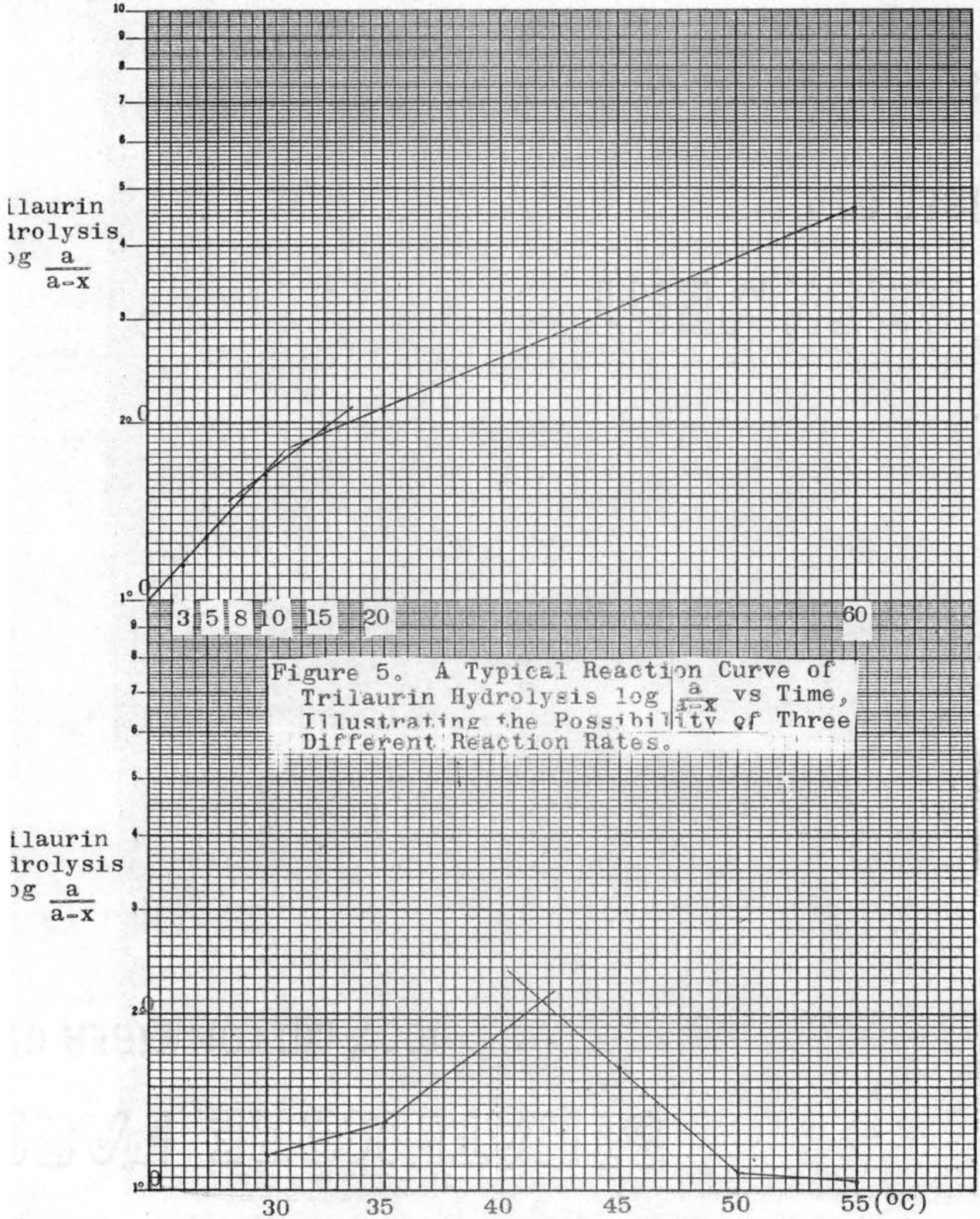

Figure 5. A Typical Reaction Curve of Trilaurin Hydrolysis log $\frac{a}{a-x}$ vs Time, Illustrating the Possibility of Three Different Reaction Rates.

Figure 6. A Graph Representing Trilaurin Hydrolysis $\frac{a}{a-x}$ vs Temperature Showing the Optimum Temperature Between 40 and 45°C.

VITA

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